

An ISA-Tab specification for protein titration data exchange

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Abstract

Data curation presents a challenge to all scientific disciplines to ensure public availability and reproducibility of experimental data. Standards for data preservation and exchange are central to addressing this challenge: the Investigation-Study-Assay Tabular (ISA-Tab) project has developed a widely used template for such standards in biological research. This paper describes the application of ISA-Tab to protein titration data. Despite the importance of titration experiments for understanding protein structure, stability, and function and for testing computational approaches to protein electrostatics, no such mechanism currently exists for sharing and preserving biomolecular titration data. We have adapted the ISA-Tab template to provide a structured means of supporting experimental structural chemistry data with a particular emphasis on the calculation and measurement of pK_a values. This activity has been performed as part of the broader pK_a Cooperative effort, leveraging data that has been collected and curated by the Cooperative members. In this article, we present the details of this specification and its application to a broad range of pK_a and electrostatics data obtained for multiple protein systems. The resulting curated data is publicly available at <http://pkacoop.org>.

Introduction

The preservation and curation of scientific research data has been a topic of discussion since the 1990s [1, 2]. In recent years, increased focus has been placed on the standards and storage needs for scientific research. In most scientific datasets, there are two broad categories of data. *Ephemeral* data is irreplaceable and cannot be regenerated while *stable* data can be reproduced from source ephemeral data [3]. The focus of this paper is the metadata associated with the ephemeral data. Metadata is a common type of data that includes the procedures necessary in order to produce the experimental data, qualitative descriptors, persons and institutions involved, and ontological tags providing semantic information for these data. Given ephemeral metadata, stable data can be regenerated via computation, simulation, or reproduction of the analysis as prescribed in the metadata. Ephemeral data, therefore, has intrinsic value for broader meta-analyses of related experimental data sets and reproduction of experimentally observed results [3]. Therefore, there is a significant need to curate data and metadata. The careful documentation and storage of ephemeral data (e.g., software used, laboratory parameters, data, etc.) is an important step toward addressing the reproducibility of stable, computational experimental outcomes.

This paper describes the development of a standard to ensure the preservation and sharing of pK_a Cooperative data. There currently exist several application-specific data curation efforts that have resulted in standards that specify how data should be preserved; e.g., the Minimum Information About a Microarray Experiment (MIAME) [4] and the Systems Biology Markup Language (SBML) [5]. However, given the diversity of experimental and computational methods used to generate protein pK_a data, we wanted to use a template that was easily extensible, available as open source, and already used in multiple applications. The schema we identified as the most applicable for this study is the Investigation-Study-Assay Tabular (ISA-Tab) format, which is an open-source standard for biological experimental data [6, 7]. ISA-Tab was originally designed for microarray data, but has been expanded to include a wide range of experimental methods relevant to protein biophysics [8], experimental data in biomedicine, nanomaterials [5], and targeted bioinformatics research on stem cells in the Stem Cell Discovery Engine (SCDE) [9]. The SCDE example is relevant to our current work extending ISA-Tab to experimental and computational research. SCDE is an analytical suite with a knowledge base that wraps experimental data using the ISA-Tab specification. This use of the ISA-Tab schema demonstrates its flexibility in configuration, generalized format of data files, as well as adaptability to biological and chemical data curation uses.

We aim to extend the ISA-Tab specification, in a manner similar to the SCDE implementation, for the measurement and calculation of acid dissociation constants (pK_a s) in proteins. The acid dissociation constants of protein residues are relevant to biochemistry and biophysics because they reveal important information about protein energetics,

stability, and function. The importance of accurately capturing these values is discussed at length in the pK_a Cooperative article which also describes the origins of our curated data [10].

Methods

ISA-Tab-formatted (meta)data is organized in a directory containing tab-delimited text files linked by prefixes in the file names [6]. These text files are divided into three categories: Investigation, Study, and Assay with the filename prefixes “i_”, “s_”, and “a_”, respectively. The tab-delimited text files can be read by a lightweight Java interpreter called the *ISAcceptor* or by a wide range of other parsers. The *ISAcceptor* interpreter parses the ISA-Tab-formatted directory of files based on XML configuration files. The configuration file can be modified by direct editing of the XML or via the *ISAcceptor configurator* software. The ISA-Tab file is static, allowing reuse across multiple datasets. The *ISAcceptor* and *ISAcceptor configurator* tools are open source tools, easily accessed through their home page [7]. We expanded the ISA-Tab configuration file by adding additional assay configurations for assays measuring chemical shift, titration curve measurements, pK_a values, and calculated electrostatic properties such as free energy. For the purposes of the pK_a Cooperative data exchange, one journal article is considered to be one investigation and an individual ISA-Tab directory of data files is created for each article curated.

Our customized ISA-Tab configuration was tested against a published journal article [11] from the García-Moreno lab that uses both NMR spectroscopy and continuum electrostatics methods to measure/calculate pK_a values for internal residues of a hyperstable variant of staphylococcal nuclease. An *ISAcceptor* view of the investigation file is shown in Figure 1 displaying information extracted from the García-Moreno journal article. The investigation file contains several important elements: (A) titles of the studies, researcher contact information, a summary of work, and date of publication; (B) pointers to the various study assay files in the **Study Assay** section; and (C) study design descriptors from several ontologies, including the Ontology for Biomedical Investigations (OBI) [12], the National Center for Biotechnology Information (NCBI) Taxonomy [13], and the National Cancer Institute (NCI) Thesaurus [14]. The left hand column in Figure 1 displays a list of all study and assay files associated with the investigation.

Figure 1. *ISAcceptor* view of investigation file. (A) titles of the studies, researcher contact information, a summary of work, and date of publication; (B) pointers to the various study assay files in the **Study Assay** section; and (C) study design descriptors from several ontologies.

An investigation generally includes several studies; individual study files can be viewed in *ISAcceptor* by selecting items from the lefthand side of the screen shown in Figure 1. An example *ISAcceptor* study file view is shown in Figure 2 and includes (A) a reference molecule ID (if available) for the protein under study and (B) a list of a list of all proteins and variants examined in the study. In the current example, the PDB ID is used as the molecule reference in the **Source Name** field; however, other IDs (GenBank, etc.) could be used as well with the appropriate references added to the **Comment[Source]** field. Mutants are described by the **Characteristics[organism]** field and given unique identifiers in the **Sample Name** field. The left hand column in Figure 2 displays a list of assay files associated with the study.

Row No.	Source Name	Comment[Source]	Characteristics[organism]	Protocol REF	Sample Name
1	3D6C	PDB-ID	Δ+PHS	sample collection	PHS
2	n/a	n/a	Δ+PHS/E122Q	sample collection	PHS/E122Q
3	n/a	n/a	Δ+PHS/L38K	sample collection	PHS/L38K
4	n/a	n/a	Δ+PHS/L38E	sample collection	PHS/L38E
5	n/a	n/a	Δ+PHS/L38E/E122Q	sample collection	PHS/L38E/E122Q
6	n/a	n/a	Δ+PHS/L38E/E122D	sample collection	PHS/L38E/E122D
7	n/a	n/a	Δ+PHS/L38E/R126Q	sample collection	PHS/L38E/R126Q
8	n/a	n/a	Δ+PHS/L38E/D77N	sample collection	PHS/L38E/D77N
9	n/a	n/a	Δ+PHS/L38D	sample collection	PHS/L38D
10	n/a	n/a	Δ+PHS/L38D/E122Q	sample collection	PHS/L38D/E122Q

Figure 2. ISA-Tab view of study file. (A) a reference molecule ID (if available) for the protein under study and (B) a list of a list of all proteins and variants examined in the study.

A study generally includes several assays; individual assay files can be view in *ISAcceptor* by selecting items from the lefthand side of the screen show in Figure 2. An example *ISAcceptor* assay file view is shown in Figure 3 and includes (A) a single measurement technology or software simulation outcome for (B) a single protein variant identified in the parent study file using (C) a specific measurement protocol to obtain (D) a pK_a value and (E) standard deviation as well as (F) other derived values. A number of ephemeral data attributes are exposed for this assay, including the protein residue (available in the **Extract Name** and the **Protocol REF** fields. The **Protocol REF** field includes an ontology concept to reference the experimental (or computational) approach used for each measurement as well as methods such as linkage analysis used to derive pK_a values from the data. Several protocols have been implemented into our configuration file, including both computational and experimental methods (described in the Results section below). In addition to capturing as much ephemeral non-reproducible data as possible; e.g., additional fitting parameters used in data analysis in **Parameter Value[Derived Experimental Data]** and associated fields such as linkage (or Hill) coefficients.

Row No.	Sample N.	Protocol REF	Assay	Extract Na.	Comment[Secondary]	Protocol REF	Para.	Cha.	Unit	Para.	Unit	Char.	Unit	Param.	Unit	Pi
1	PHS/L38E	titration	NMR	Glul38	Comparison to NMR	Linkage Analysis	7.0	0.1	data_1529j	0.0	ΔpKa	n/a	data_1529j	0.8	SBQ Hill coefficient	
2	PHS/L38E	titration	NMR	His8		NMR spectroscopy	6.5	n/a	data_1529j	0.0	ΔpKa	n/a	data_1529j	1.0	SBQ Hill coefficient	
3	PHS/L38E	titration	NMR	His121		NMR spectroscopy	5.7	n/a	data_1529j	0.0	ΔpKa	n/a	data_1529j	1.0	SBQ Hill coefficient	
4	PHS/L38E	titration	NMR	D19	Asp/Glu residue position	NMR spectroscopy	<2.5	0.1	data_1529j	0.0	ΔpKa	0.1	data_1529j	n/a	SBQ Hill coefficient	
5	PHS/L38E	titration	NMR	D21	Asp/Glu residue position	NMR spectroscopy	6.6	0.1	data_1529j	0.0	ΔpKa	0.1	data_1529j	n/a	SBQ Hill coefficient	
6	PHS/L38E	titration	NMR	D40	Asp/Glu residue position	NMR spectroscopy	3.9	0.1	data_1529j	0.0	ΔpKa	0.1	data_1529j	n/a	SBQ Hill coefficient	
7	PHS/L38E	titration	NMR	D77	Asp/Glu residue position	NMR spectroscopy	<2.5	0.1	data_1529j	0.0	ΔpKa	0.1	data_1529j	n/a	SBQ Hill coefficient	
8	PHS/L38E	titration	NMR	D83	Asp/Glu residue position	NMR spectroscopy	<2.5	0.1	data_1529j	0.0	ΔpKa	0.1	data_1529j	n/a	SBQ Hill coefficient	
9	PHS/L38E	titration	NMR	D95	Asp/Glu residue position	NMR spectroscopy	2.3	0.1	data_1529j	0.1	ΔpKa	0.1	data_1529j	n/a	SBQ Hill coefficient	
10	PHS/L38E	titration	NMR	D143	Asp/Glu residue position	NMR spectroscopy	3.8	0.1	data_1529j	0.0	ΔpKa	0.1	data_1529j	n/a	SBQ Hill coefficient	
11	PHS/L38E	titration	NMR	D146	Asp/Glu residue position	NMR spectroscopy	3.9	0.1	data_1529j	0.0	ΔpKa	0.1	data_1529j	n/a	SBQ Hill coefficient	
12	PHS/L38E	titration	NMR	E10	Asp/Glu residue position	NMR spectroscopy	3.1	0.1	data_1529j	0.2	ΔpKa	0.1	data_1529j	n/a	SBQ Hill coefficient	
13	PHS/L38E	titration	NMR	E43	Asp/Glu residue position	NMR spectroscopy	4.4	0.1	data_1529j	0.0	ΔpKa	0.1	data_1529j	n/a	SBQ Hill coefficient	
14	PHS/L38E	titration	NMR	E52	Asp/Glu residue position	NMR spectroscopy	3.6	0.1	data_1529j	0.1	ΔpKa	0.1	data_1529j	n/a	SBQ Hill coefficient	
15	PHS/L38E	titration	NMR	E57	Asp/Glu residue position	NMR spectroscopy	3.6	0.1	data_1529j	0.1	ΔpKa	0.1	data_1529j	n/a	SBQ Hill coefficient	
16	PHS/L38E	titration	NMR	E67	Asp/Glu residue position	NMR spectroscopy	3.8	0.1	data_1529j	0.1	ΔpKa	0.1	data_1529j	n/a	SBQ Hill coefficient	
17	PHS/L38E	titration	NMR	E73	Asp/Glu residue position	NMR spectroscopy	3.4	0.1	data_1529j	0.1	ΔpKa	0.1	data_1529j	n/a	SBQ Hill coefficient	
18	PHS/L38E	titration	NMR	E75	Asp/Glu residue position	NMR spectroscopy	3.3	0.1	data_1529j	0.1	ΔpKa	0.1	data_1529j	n/a	SBQ Hill coefficient	
19	PHS/L38E	titration	NMR	E101	Asp/Glu residue position	NMR spectroscopy	3.9	0.1	data_1529j	0.1	ΔpKa	0.1	data_1529j	n/a	SBQ Hill coefficient	
20	PHS/L38E	titration	NMR	E122	Asp/Glu residue position	NMR spectroscopy	3.8	0.1	data_1529j	-0.1	ΔpKa	0.1	data_1529j	n/a	SBQ Hill coefficient	
21	PHS/L38E	titration	NMR	E129	Asp/Glu residue position	NMR spectroscopy	3.9	0.1	data_1529j	0.1	ΔpKa	0.1	data_1529j	n/a	SBQ Hill coefficient	
22	PHS/L38E	titration	NMR	E135	Asp/Glu residue position	NMR spectroscopy	3.8	0.1	data_1529j	0.1	ΔpKa	0.1	data_1529j	n/a	SBQ Hill coefficient	
23	PHS/L38E	titration	NMR	E142	Asp/Glu residue position	NMR spectroscopy	4.5	0.1	data_1529j	0.0	ΔpKa	0.1	data_1529j	n/a	SBQ Hill coefficient	

Figure 3. ISA-Tab view of assay file. (A) A single measurement technology or software simulation outcome for (B) a single protein variant identified in the parent study file using (C) a specific measurement protocol to obtain (D) a pK_a value and (E) standard deviation as well as (F) other derived values.

Results and discussion

We tested our extended ISA-Tab data-sharing format by curating information from 20 published manuscripts with protein titration data. These manuscripts included NMR spectroscopy [11, 15–29], UV circular dichroism [30, 31], and continuum electrostatics calculations [11, 18, 21, 24, 26–29, 31–33]. To increase the diversity of the test dataset and test generalizability beyond pK_a values, we also included electrochemistry data of heme proteins [32]. The curation of both experimental and computational data is an important aspect of this work. The pK_a Cooperative recently led a blind prediction challenges for pK_a data given small amounts of seed experimental information [34, 35]. Meaningful analysis and comparison [10, 36] between computational predictions requires a standardized data exchange format that captures relevant metadata. The curated ISA-Tab-formatted versions of these articles as well as the ISA-Tab configuration files can be downloaded from (<https://pkacoop.org>). The Supporting Information for this manuscript contains a ZIP file of the ISA-Tab materials as well as step-by-step instructions for using the files with the *ISAcceptor* software. This curated data is managed via GitHub due to its open availability and participant-sourced revision process.

In future work, we hope to expand the set of curated data and supported assays as well as extend curation to include pK_a s measured in small molecule compounds such as pharmaceuticals [37]. Future work will also include automatic formatting of CSV spreadsheets from existing data tables into an ISA-Tab compliant format to decrease curation time requirements for current and future research. As minimal requirements for pK_a data sharing, we recommend that the pK_a parameter values, statistical characteristics of measurements and fits (e.g., standard errors), fitting models, assay types, residues measured or continuum electrostatics model used be included in reported data. We hope that the ISA-Tab pK_a data sharing format will enjoy adoption within and beyond the pK_a Cooperative and serve as a starting point for broader adoption of informatics standards by the molecular biophysics community.

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Supporting information

The ISA-Tab-formatted data, described above, are available for download from the pK_a Co-operative GitHub web page: <http://pkacoop.org>. A ZIP format is also provided as Supporting Information to this manuscript. The ZIP archive includes the ISA-Tab configuration file described in this paper as well as the individual datasets. The *ISAcreeator* application full version is required to view the ISA-Tab files through the customized user interface rather than as spreadsheets; this software is freely available from <http://www.isa-tools.org/software-suite/>. After creating an account in *ISAcreeator*, users can add the `isaconfigPChem` directory to the list of available *ISAcreeator* configurations. Alternatively, users can copy this directory to their *ISAcreeator* installation directory (under Configurations) so that it will be available by default. The provided pK_a Co-operative ISA-Tab (found in the `isa-tab-data` directory of Supporting Information) can be loaded through the main menu of the *ISAcreeator* software.